LIVER AND BILIARY DISEASE

Underdiagnosis of hereditary haemochromatosis: lack of presentation or penetration?

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Gut 2002;**51**:108–112

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Accepted for publication 4 September 2001

Background: The majority of hereditary haemochromatosis (HH) patients are homozygous for the C282Y mutation in the HFE gene. We have demonstrated a homozygote frequency of 1 in 83 for the C282Y mutation in a retrospective analysis of Irish neonates. However, a fully developed phenotype is not observed at the same frequency clinically, suggesting that a large proportion of Irish HH patients may remain undiagnosed.

Aims: To determine whether underdiagnosis of HH results from the non-specific nature of early symptoms or incomplete penetrance of the C282Y mutation.

Methods: Seventy nine C282Y homozygous individuals identified from family screening for HH and 30 HH probands were investigated. Non-specific symptoms (fatigue, arthropathy, and impotence) and their association with iron indices (transferrin saturation and serum ferritin) and hepatic iron deposition were analysed.

Results: We found that 78% of men (mean age 42 years) and 36% of women (mean age 39 years) who were identified as C282Y homozygotes following family screening had iron overload, as defined by a transferrin saturation ⩾52% combined with a serum ferritin ⩾300 µg/l for men and ⩾200 µg/l for women. The frequency of reports of non-specific symptoms in those individuals with iron overload was not significantly different from those who did not have iron overload.

Conclusions: Our findings indicate that underdiagnosis of HH may be due to the non-specific nature of early symptoms and less frequently to the incomplete penetrance of the C282Y mutation.

Hereditary haemochromatosis (HH) is the most common autosomal recessive disease in those of North European descent, with a carrier frequency estimated between 1:8 and 1:10 and a homozygote frequency between 1:200 and 1:400.[1] The majority of patients are homozygous for the C282Y mutation in the HFE gene but a small proportion are compound heterozygous for C282Y and H63D or homozygous for the H63D mutation.[2–7] Some individuals have no identifiable HFE mutation or are heterozygotes for C282Y or H63D suggesting that other additional HFE mutations may be involved.[8–10]

There is considerable variation in the penetrance of the HFE mutations but homozygosity for the C282Y mutation is the most penetrant, leading to iron overload in at least 60% of affected individuals.[11] Although environmental factors may sometimes explain the differences in variability of iron accumulation and associated clinical presentation, it is more likely that genes other than HFE and/or other HFE mutations are responsible for this phenotypic variation.[12] Animal studies have already demonstrated that other genes involved in iron metabolism can modify the HH phenotype and it is hypothesised that loss of function mutations or polymorphisms in these genes could ameliorate or exacerbate the HH phenotype.[13]

We have demonstrated that 93% of Irish HH patients are homozygous for the C282Y mutation and we have also identified an allele frequency of 11% in Irish neonates, indicating a homozygote frequency of 1 in 83 for this mutation.[14 15] The relationship between HH genotype and phenotype in the Irish population has not yet been examined but our finding of a C282Y homozygote frequency of 1 in 83 is not reflected in the clinical setting. This may suggest that a large proportion of Irish HH homozygotes remain undiagnosed. This underdiagnosis may result from lack of awareness of the disease, its long latency period, and its non-specific symptoms, or simply incomplete penetrance.[16 17]

Consequently, the purpose of this study was to determine whether underdiagnosis or incomplete penetrance could explain the discrepancy between genetic prevalence (genotype) and clinical expression (phenotype). In particular, non-specific symptoms (fatigue, arthropathy, and impotence) and their association with iron indices (transferrin saturation and serum ferritin) were analysed. Seventy nine C282Y homozygous relatives identified from family screening were selected without reference to their health status and can be considered to be clinically unselected. This approach overcomes ascertainment bias that results in an overestimate of disease expression when patients are detected based on symptomatology. Furthermore, this approach can be considered to be a substitute or surrogate method for population screening as the identification of 79 C282Y homozygous individuals from population screening would have necessitated screening at least 7000 individuals based on a homozygote frequency of 1 in 83 for this mutation in Ireland.

MATERIALS AND METHODS

Probands

Thirty unrelated patients diagnosed with HH were studied and comprised 22 males (mean age at diagnosis 47 years, range 24–72) and eight females (mean age at diagnosis 39 years, range 30–60). The diagnosis was made on the basis of clinical history, physical examination, persistently raised iron indices, and hepatic iron deposition. All patients were homozygous for the C282Y mutation. The following variables were analysed: sex, age at diagnosis, transferrin saturation (%), serum ferritin, liver enzymes (alanine aminotransferase (ALT)

Abbreviations: HH, hereditary haemochromatosis; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

Table 1 Age and iron indices in male and female probands and in the C282Y homozygotes identified through family screening

Variable	Male probands (n=22)	Male C282Y homozygotes (n=40)	Female probands (n=8)	Female C282Y homozygotes (n=39)
Age (y)	47 (11)	41 (14)	39 (10)	38 (15)
Transferrin saturation (%)	80 (14)	75 (13)	80 (16)	64 (21)
Serum ferritin (µg/l)	1277 (860)	889 (891)	732 (357)	222 (224)

Results are means (SD).

Table 2 Comparison of symptomatology at diagnosis or screening and iron indices in male probands and in C282Y homozygotes identified through family screening with iron overload, as defined by transferrin saturation ⩾52% and serum ferritin ⩾300 µg/l

Variable	Probands	Identified C282Y homozygotes with iron overload	p Value
Age (y)	48 (10) (n=21)	42 (13) (n=31)	0.082
Transferrin saturation (%)	81 (14) (n=21)	80 (10) (n=31)	0.620
Serum ferritin (µg/l)	1327 (846) (n=21)	1049 (930) (n=31)	0.059
Fatigue	14/19 (74%)	17/31 (55%)	0.183
Arthropathy	9/19 (47%)	11/31 (35%)	0.405
Impotence	2/15 (13%)	5/26 (19%)	1.00

Results are means (SD).

and aspartate aminotransferase (AST)), and the presence or absence of fatigue, arthropathy, and impotence at diagnosis.

Subjects identified from family screening

Seventy nine C282Y homozygous individuals were identified from family screening. These were from 46 families and consisted of 43 siblings, 25 children, three parents, two cousins, five nieces/nephews, and one grandchild. There were 40 males (mean age at screening 41 years (17–72) and 39 females (mean age at screening 38 years (15–69)).

The following variables were analysed: sex, age at screening, relationship to proband, HFE status, transferrin saturation (%), serum ferritin, liver enzymes (ALT and AST), and the presence or absence of fatigue, arthropathy, and impotence at screening.

Serum iron indices

Fasting serum iron concentration and total iron binding capacity were measured using a colorimetric assay on a Beckman CX7 analyser. Transferrin saturation was then calculated as serum iron/total iron binding capacity×100. Transferrin saturation was considered to be elevated when ⩾52%. Fasting serum ferritin was measured using a microparticle enzyme immunoassay on an Abbott IMX analyser. Iron overload was considered to be present when serum ferritin was ⩾300 µg/l for men and ⩾200 µg/l for women.

Liver enzymes

ALT and AST were considered to be elevated when >40 IU/l .

Identification of HFE mutations

DNA was extracted from whole blood or from blood spots on Guthrie cards and amplified for the C282Y and H63D mutations, as previously described.[14] Homozygosity for C282Y was confirmed using the primer described by Jeffrey and colleagues.[18]

Hepatic histology

Liver biopsy sections were stained with Perls' Prussian blue stain for iron. The amount of stainable iron in hepatocytes was graded 0–4.[19] The presence or absence of liver architectural changes, including inflammation, fibrosis, and cirrhosis were also noted.

Statistical analysis

All data were entered on an SPSS version 8.0 for Windows database. Values are presented as means (SD). Comparisons between groups were made using χ^2 analysis or Fisher's exact test for symptomatology; the Mann-Whitney U test for serum ferritin; and the independent *t* test for per cent transferrin saturation and age. All p values are two tailed. A p value of ⩽0.05 was considered significant.

RESULTS

Iron indices

For the purposes of this study, iron overload was considered to be present when fasting transferrin saturation was ⩾52% combined with a serum ferritin ⩾300 µg/l for males or ⩾200 µg/l for females.

Probands

The study compared 79 C282Y homozygous relatives (40 men and 39 women) with 30 (22 men and eight women) probands (table 1).

Ninety five per cent of male probands (21/22) had iron overload, as defined by the above criteria. The remaining male proband (1/22) had a transferrin saturation of 60% and a ferritin of 214 µg/l aged 24 years, and was identified during a routine medical screen when he was found to have mildly abnormal liver function tests (alkaline phosphatase 203 IU/l and ALT 54 IU/l).

Eighty eight per cent (7/8) of female probands had iron overload, as defined by the above criteria. The remaining female proband (1/8) had a transferrin saturation of 69% and a serum ferritin of 174 µg/l at 60 years. She was not initially diagnosed at this centre and the reason for her initial presentation is not available.

Identified homozygotes

The majority of male C282Y homozygotes identified by family screening—that is, 78% (31/40)—had iron overload while 36% (14/39) of females had iron overload. Age, iron indices, and symptomatology of those found to be C282Y homozygotes through family screening were compared with those of the proband cohorts (tables 2, 3). Mean age of the probands (male and female) was not significantly different from that in the

Table 3 Comparison of symptomatology at diagnosis or screening and iron indices in female probands and in female C282Y homozygotes identified through family screening with iron overload, as defined by transferrin saturation ⩾52% and serum ferritin ⩾200 µg/l

Variable	Probands	Identified C282Y homozygotes with iron overload	p Value
Age (y)	36 (5) (n=7)	39 (15) (n=14)	0.600
Transferrin saturation (%)	82 (17) (n=7)	72 (13) (n=14)	0.173
Serum ferritin (µg/l)	811 (299) (n=7)	419 (248) (n=14)	0.003
Fatigue	6/6 (100%)	6/14 (43%)	0.041
Arthropathy	2/7 (29%)	3/14 (21%)	1.00

Results are means (SD).

Table 4 Comparison of symptomatology at diagnosis or screening and iron indices in male C282Y homozygotes identified through family screening with iron overload compared with those without iron overload

Variable	Identified C282Y homozygotes with iron overload	Identified C282Y homozygotes without iron overload	p Value
Age (y)	42 (13) (n=31)	36 (16) (n=9)	0.246
Transferrin saturation (%)	80 (10) (n=31)	60 (13) (n=9)	0.000
Serum ferritin (µg/l)	1049 (930) (n=31)	333 (418) (n=9)	0.0002
Fatigue	17/31 (55%)	3/9 (33%)	0.45
Arthropathy	11/31 (35%)	3/8 (37.5%)	1.00
Impotence	5/26 (19%)	1/9 (11%)	1.00

Results are means (SD).

Table 5 Comparison of symptomatology at diagnosis or screening and iron indices in female C282Y homozygotes identified through family screening with iron overload compared with those without iron overload

Variable	Identified C282Y homozygotes with iron overload	Identified C282Y homozygotes without iron overload	p Value
Age (y)	39 (15) (n=14)	37 (15) (n=25)	0.740
Transferrin saturation (%)	72 (13) (n=14)	59 (23) (n=25)	0.056
Serum ferritin (µg/l)	419 (248) (n=14)	112 (104) (n=24)	0.000
Fatigue	6/14 (43%)	7/25 (28%)	0.481
Arthropathy	3/14 (21%)	1/25 (4%)	0.123

Results are means (SD).

identified homozygotes (male and female). Nine of 40 identified male C282Y homozygotes did not have iron overload, six of these nine (67%) had a transferrin saturation ⩾52%, and one had a serum ferritin level ⩾300 µg/l. Twenty five women did not have iron overload, 16 (64%) of these had transferrin saturation ⩾52%, and three had elevated serum ferritin (226 µg/l at age 36 years, 312 µg/l at age 68 years, and 483 µg/l at age 69 years). Age, iron indices, and symptomatology in male and female patients who were found to be C282Y homozygotes and expressed HH were compared with those identified homozygotes who did not have iron overload (tables 4, 5). Mean age of the identified expressing homozygotes (male and female) was not significantly different than the mean age of the non-expressing homozygotes (male and female).

Liver enzymes

ALT and AST levels at the time of diagnosis or screening were available for 54 individuals (16 probands and 38 identified C282Y homozygotes). Of the 16 probands, 12 reported fatigue at the time of diagnosis, eight of whom had raised ALT levels and five of these eight also had raised AST levels. Nineteen of the 38 patients found to be homozygotes following family screening reported fatigue at the time of screening, eight of whom had raised ALT; three of these eight also had raised AST levels. Fourteen of the 19 identified homozygotes who reported fatigue at the time of screening had elevated iron indices, seven of whom had raised ALT and three of these seven had raised AST levels. No significant associations were observed with regard to elevated ALT or AST levels and fatigue in the group of 54 individuals overall, nor was there a positive association between elevated iron indices (probands and identified homozygotes with elevated iron indices) and fatigue.

Symptomatology

Males

Fatigue was reported by 74% (14/19) of male probands and 55% (17/31) of the expressing iron overloaded male homozygotes (p=0.183): corresponding values for arthropathy were 47% (9/19) compared with 35% (11/31) (p=0.405), and for impotence, 13% (2/15) compared with 19% (5/26) (p=1.00).

Three of nine (33%) men who were C282Y homozygous but did not have iron overload had fatigue compared with 17/31 (55%) in the expressing group (p=0.45). Three of eight without iron overload had arthropathy compared with 11/31 in the expressing group (p=1.00). One of nine without iron overload

Table 6 Hepatic iron deposition in male and female probands, in C282Y homozygotes identified through family screening with iron overload, and in C282Y homozygotes identified through family screening without iron overload

Cohort	Hepatic iron deposition				
	0	1+	2+	3+	4+
Males					
Probands (n=17)	0	1	2	8	6
Identified with iron overload (n=20)	0	5	4	9	2
Identified without iron overload (n=4)	0	3	1	0	0
Females					
Probands (n=6)	0	1	1	3	1
Identified with iron overload (n=10)	1	1	1	5	2
Identified without iron overload (n=7)	1	2	3	1	0

was impotent at screening compared with 5/26 in the expressing cohort (p=1.00)

Females

All female probands complained of fatigue compared with 43% (6/14) of the expressing female cohort (p=0.041). Twenty nine per cent of probands (2/7) and 21% (3/14) of the expressing homozygotes had arthropathy (p=1.00).

Seven of 24 (28%) identified females without iron overload had fatigue compared with six of 14 (43%) in the expressing cohort (p=0.481), and one of 25 (4%) without iron overload had arthropathy at the time of screening compared with three of 14 (21%) in the expressing group (p=0.123).

Liver histology

Information on hepatic iron deposition was available for 23 iron overloaded probands, 30 individuals identified from family screening with elevated iron indices, and 11 individuals without elevations in both transferrin saturation and serum ferritin. These 11 individuals underwent liver biopsy because of elevated transferrin saturation in 10 of 11 cases or elevated serum ferritin in one case (table 6).

There were no significant differences in the frequency of reports of fatigue and arthropathy in males and females and impotence in males when those with hepatic iron deposition ⩾3+ were compared with those who had <3+ iron deposition for both the proband and identified cohorts. It was not possible to compare symptomatology in the non-elevated iron indices group with respect to hepatic iron deposition because the numbers were too small.

Information on the presence or absence of inflammation, fibrosis, and cirrhosis was available for 17 probands (14 male and three female), 16 identified homozygotes with elevated iron indices (12 male and 4 female), and six identified homozygotes without elevated iron indices. No significant differences were observed in the incidence of inflammation, fibrosis, or cirrhosis in the proband cohorts (male and female) compared with the elevated iron indices cohorts (male and female).

DISCUSSION

This study attempted to address the apparent discrepancy between homozygosity for the HFE C282Y mutation and disease penetrance. The study used a surrogate method for estimation of the penetrance of the C282Y mutation, whereby 79 C282Y homozygous individuals identified or discovered from family screening were screened for phenotypic expression of the disease. Identification of this number of homozygotes by population screening strategies would have involved screening between 7000 and 9000 individuals depending on geographic location. Subsequently, these identified homozygotes were compared with a group of C282Y homozygous probands. Expression of HH, as defined by a transferrin saturation ⩾52% and serum ferritin ⩾300 μg/l for males and ⩾200 μg/l for females and its association with fatigue, arthropathy, and impotence, were specifically investigated. A cut off value of ⩾52% was chosen as this value equates to a transferrin saturation of ⩾45% calculated when transferrin is detected immunochemically, and identifies 98% of affected individuals while producing few false positive results.[20 21]

Our data showed that 78% of males (mean age 42 years) and 36% of females (mean age 39 years) who were identified as C282Y homozygotes following family screening were found to have iron overload. The fact that only 36% of identified female C282Y homozygotes expressed HH is probably a reflection of the protection afforded to women via menstruation and pregnancy.

Interestingly, if an elevated transferrin saturation of ⩾52% had been used as the only screening tool, 93% of male and 77% of female C282Y homozygotes discovered through family screening could be said to be exhibiting early phenotypic expression.

Although the frequency of fatigue in female probands was significantly different from the identified expressing cohort, the overall frequency of reports of non-specific symptoms (fatigue, arthropathy, and impotence) in male and female individuals with iron overload (probands and expressing homozygotes) was not significantly different from those identified homozygotes who did not have iron overload. It is known that women report fatigue more frequently than men but the pathogenesis of fatigue in HH and indeed other chronic liver diseases is still poorly understood.[22 23]

Iron overload was found in the presence of fatigue (55% males and 43% females), arthropathy (35% males and 21% females), and impotence (19% of males), which shows that many identified homozygotes are in fact symptomatic, with frequently unrecognised early non-specific symptoms that can develop into life threatening disease. This emphasises the importance of family screening and indeed the requirement on the part of the physician to have a high index of suspicion in the presence of non-specific symptoms when a patient attends for investigation of these symptoms. Our findings support a recent study that examined the prevalence of disease related conditions defined as cirrhosis, fibrosis, elevated aminotransferase levels, and radiologically confirmed haemochromatotic arthropathy in 214 C282Y homozygous relatives.[24] The results demonstrated that a substantial number of homozygous relatives (52% of males >40 years and 16% of females >50 years) have conditions related to HH that have yet to be clinically detected. These data also support our conclusions regarding the importance of conducting thorough family screening.

Conversely, our finding that a substantial proportion of the 78% of male and 36% of female identified homozygotes that had iron overload were in fact asymptomatic highlights the

importance of considering evaluation of iron status as part of routine adult health care.[16] This finding may also indicate that asymptomatic individuals identified through biochemical screening may have milder clinical manifestations compared with probands and that a proportion may remain without serious clinical symptoms even if untreated.[25]

One of the problems associated with penetrance studies is the question of what constitutes a diagnosis of HH. A diagnosis of HH can be based on clinical symptomatology such as pigmentation, diabetes, and liver disease, on biochemical expression, as defined by elevations in transferrin saturation and serum ferritin, or as defined by an elevation in transferrin saturation only, and by homozygosity for the C282Y mutation.[25] There may be considerable discrepancies in estimations of disease penetrance depending on whether penetrance is based on clinical symptomatology or on biochemical parameters. We report here a disease penetrance based on biochemical parameters with respect to homozygosity for C282Y in 78% of men and in 36% of women who were discovered through family screening. In conclusion, our results indicate that underdiagnosis of HH may in fact be due to the non-specific nature of early symptoms and less frequently to the incomplete penetrance of the C282Y mutation.

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REFERENCES

1 **Edwards CQ**, Kushner JP. Screening for hemochromatosis. *N Engl J Med* 1993;**328**:1616–20.
2 **Feder JN**, Gnirke A, Thomas W, *et al*. A novel MHC-class 1 like gene is mutated in patients with hereditary hemochromatosis. *Nat Genet* 1996;**13**:399–408.
3 **Jazwinska EC**, Cullen LM, Bushfield F, *et al*. Hemochromatosis and HLA-H. *Nat Genet* 1996;**14**:249–51.
4 **Jouannolle AM**, Gandon G, Jezequel M, *et al*. Hemochromatosis and HLA-H. *Nat Genet* 1996;**14**:251–2.
5 **The UK Hemochromatosis Consortium**. A simple test identifies 90% of UK patients with hemochromatosis. *Gut* 1997;**41**:841–4.
6 **Fairbanks VF**, Brandhagen DJ, Thibodeau SN, *et al*. H63D is a haemochromatosis associated allele. *Gut* 1998;**43**:441–4.
7 **Sham RL**, Chin-Yih O, Cappuccio J, *et al*. Correlation between genotype and phenotype in hereditary hemochromatosis:analysis of 61 cases. *Blood Cells Mol Dis* 1997;**23**:314–20.
8 **Mura C**, Raguenes O, Ferec C. HFE mutations analysis in 711 hemochromatosis probands :evidence for S65C implication in mild form of hemochromatosis. *Blood* 1999;**93**:2502–5.
9 **Wallace DF**, Dooley JS, Walker AP. A novel mutation of HFE explains the classical phenotype of genetic haemochromatosis in a C282Y heterozygote. *Gastroenterology* 1999;**116**:1409–12.
10 **Barton JC**, Sawada-Hirai R, Rothenberg BE, *et al*. Two novel missense of the HFE gene (I105T and G93R) and identification of the S65C mutation in Alabama hemochromatosis probands. *Blood Cells Mol Dis* 1999;**25**:147–55.
11 **Adams P**, Brissot P, Powell L. EASL International Consensus Conference on Haemochromatosis. *J Hepatol* 2000;**33**:485–504.
12 **Anderson GJ**, Powell LW. Of metals, mice, and men: what animal models can teach us about body iron loading. *J Clin Invest* 2000;**105**:1185–6.
13 **Levy JE**, Montross LK, Andrews NC. Genes that modify the hemochromatosis phenotype in animals. *J Clin Invest* 2000;**105**:1209–16.
14 **Ryan E**, O'Keane C, Crowe J. Hemochromatosis in Ireland and HFE. *Blood Cells Mol Dis* 1998;**24**:428–32.
15 **Byrnes V**, Ryan E, Mayne P, *et al*. Neonatal screening for the HFE mutations in the Irish population. *Gut* 1999;**1**:527A.
16 **Powell LW**, George DK, McDonnell SM, *et al*. Diagnosis of hemochromatosis. *Ann Intern Med* 1998;**129**:925–31.
17 **Adams PC**. Non-expressing homozygotes for C282Y hemochromatosis: minority or majority of cases? *Mol Genet Metab* 2000;**71**:81–6.
18 **Jeffrey GP**, Chakrabarti S, Hegele RA, *et al*. Polymorphism in intron 4 of HFE may cause overestimation of C282Y homozygote prevalence in haemochromatosis. *Nat Genet* 1999;**22**:325–6.
19 **Scheuer PJ**, Williams R, Muir AR. Hepatic pathology in relatives of patients with hemochromatosis. *J Pathol Bacteriol* 1962;**84**:53–64.
20 **McCullen MA**, Crawford DHG, Dimeski G, *et al*. Why there is discordance in reported decision thresholds for transferring saturation when screening for hereditary hemochromatosis. *Hepatology* 2000;**32**:1410.
21 **McLaren CE**, McLachlan GJ, Halliday JW, *et al*. Distribution of transferrin saturation in an Australian population: relevance to the early diagnosis of hemochromatosis. *Gastroenterology* 1998;**114**:543–9.
22 **Adams PC**, Deugnier Y, Moirand R, *et al*. The relationship between iron overload, clinical symptomatology and age in 410 patients with genetic hemochromatosis. *Hepatology* 1997;**25**:162–6.
23 **Moirand R**, Adams PC, Bichler V, *et al*. Clinical features of genetic hemochromatosis in women compared to men. *Ann Intern Med* 1997; **127**:105–10.
24 **Bulaj ZJ**, Ajioka RS, Phillips JD, *et al*. Disease related conditions in relatives of patients with hemochromatosis. *N Engl J Med* 2000;**343**:1529–35.
25 **Burke W**, Press N, McDonnell SM. Hemochromatosis: genetics helps to define a multifactorial disease. *Clin Genet* 1998; **54**:1–9.